

# Clinical significance of Matrilin-3 gene polymorphism in Egyptian patients with primary knee osteoarthritis

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## Abstract

**Objective:** Osteoarthritis (OA) is a multifactorial, degenerative, and inflammatory disorder of joints causing damage of the articular cartilage, formation of osteophytes, and eburnation of the subchondral bone. Matrilin-3 (*MATN-3*) is a non-collagenous oligomeric extracellular matrix protein (ECM), which is the smallest member of the matrilin family. This study was conducted to identify the potential association and clinical significance of *MATN-3* rs8176070 (SNP6) polymorphism in a series of Egyptian patients with primary knee OA.

**Material and Methods:** Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine genotypes of *MATN-3* SNP6 for 50 primary knee OA patients in addition to 50 healthy subjects of the same sex and age range. Full history was obtained from OA patients, followed by clinical examination, together with clinical assessment of the severity of knee OA using Lequesne Algofunctional Index score and radiological grading using the Kellgren-Lawrence grade scale (KL).

**Results:** With regard to genotypes of *MATN-3* gene SNP6 (rs8176070), a statistically significant difference between OA patients and healthy control subjects was found for the B\b genotype and b allele ( $p=0.046$  and  $0.042$  respectively), with the prevalence being higher in OA patients with a high risk to develop OA (Odds Ratio [OR]=2.250, 95% CI=1.011-5.008). Patients with the B\b genotype had worse clinical and radiological findings than those with B\b and b\b genotypes.

**Conclusion:** The investigated polymorphism in the *MATN-3* gene may reflect the risk and severity of knee OA in Egyptian patients, particularly with the B\b genotype.

**Keywords:** Matrilin-3, osteoarthritis, lequesne algofunctional index score, Kellgren-Lawrence grade, restriction fragment length polymorphism

## Introduction

Primary osteoarthritis (OA) is a widespread chronic degenerative joint disorder with subsequent focal degeneration and abrasion of the articular cartilage, subchondral bone changes, and formation of osteophytes on the joint surface (1). Secondary inflammation may occur, resulting in pain, limitation of movement, and disability but without systemic effects (2, 3).

The initiating events in OA are not completely understood. Genetic factors appear to affect the risk of developing primary OA (4). Several genome-wide association studies (GWAS) have tried to detect OA susceptibility genes and demonstrated single-nucleotide polymorphisms (SNPs) in various genes related to an increased risk of knee OA, including collagen genes (*COL1A1*, *COL2A1*, *COL9A1*, and *COL11A2*) as well as genes encoding interleukin-1 receptor (IL1R), transforming growth factor- $\beta$ 1 (*TGF $\beta$ 1*), cartilage matrix protein 1 (*CMP1*), tissue inhibitor of metalloproteinase 3 (*TIMP3*), insulin-like growth factor-1 (*IGF1*), bone morphogenetic protein (*BMP*), vitamin D receptor (*VDR*), aggrecan-1 (*AGC1*), secreted frizzled-related protein 3 (*FRZB*), growth differentiation factor 5 (*GDF5*), and cyclooxygenase-2 (*COX-2*) (5-7).

Matrilin is a protein family that has four members termed matrilin-1-4, consisting of von Willebrand factor A domains, epidermal growth factor-like domains, and a C-terminal coiled-coil domain (8). The smallest family member Matrilin-3 (*MATN-3*) interacts with collagen fibrils, multiple proteoglycans, and other glycoproteins; thus, it plays a main role in the formation of a filamentous matrix network. It is encoded by the *MATN-3* gene, which is present on the short arm of the chromosome 2 region 2p24-p23 (9).

The regulation of *MATN-3* expression is necessary for preservation of the cartilage extracellular matrix (ECM) microenvironment (10). Mutations in *MATN-3* have been identified in the pathogenesis of many disorders, including hereditary microepiphyseal dysplasia and spondyloepimetaphyseal dysplasia, and predispose individuals to develop OA (11-13).



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In this case-control study, we chose a subtype of OA, knee OA, and aimed to identify the possible association and clinical significance of *MATN-3* rs8176070 (SNP6) polymorphism in a series of Egyptian patients with primary knee OA.

## Material and Methods

### Study approval

The study was conducted in accordance with the guidelines of the Declaration of Helsinki (World Medical Association, 2008), and the local ethics committee of Benha University School of Medicine approved the study protocol (14). Informed written consent was obtained from all the participants before they were enrolled in the study.

The present case-control study included 50 primary knee OA patients (Group I) fulfilling the American College of Rheumatology (ACR) classification criteria of knee OA who attended or were admitted to the rheumatology, rehabilitation, and physical medicine outpatients' clinic and inpatients' department of Benha University hospitals during the period from February 2015 to May 2015, together with 50 age- and sex-matched apparently healthy controls (Group II) with no symptoms or signs on clinical examination or radiographic changes indicative of knee OA or other joint disease (15). The practical part of the study was done at the Clinical and Chemical Pathology Department, Benha School of Medicine.

Patients were excluded from the study if they had one or more of the following: 1) isolated patello-femoral OA; 2) secondary OA due to trauma or deformity; metabolic disorders such as diabetes mellitus, thyroid disorders, or Cushing syndrome; or renal failure or were undergoing dialysis; 3) infectious disorders such as septic arthritis, viral arthritis, or fungal arthritis; 4) inflammatory arthritis such as rheumatoid arthritis, systemic lupus erythematosus, and sero-negative arthritis; 5) malignancy; 6) bilateral knee replacements; or 7) relatives included in the study.

Detailed histories of all the patients were obtained, and general examination and complete knee joint examination were performed. The body mass index (BMI) was calculated for all subjects included in the study as follows: the body mass was divided by the square of the body height. Clinically, the standardized Lequesne Algofunctional Index was used to evaluate disease severity through the assessment of pain/discomfort, maximum distance walked, and activities of daily living, with a maximum index score of 24 (16).

The affected knee was chosen on the basis of subjective symptoms and clinical signs, and X-radiography in the anteroposterior view in the weight-bearing position was performed, followed by graded according to the Kellgren-Lawrence (KL) grading system (17). Patients were classified as mild (if the KL grade was 1 or 2) and severe (if the KL grade was 3 or 4).

In total, 2 mL of venous blood was collected in EDTA-containing tubes from all subjects who participated in the study and stored immediately at -40°C till the time of DNA extraction.

Analysis of *MATN3* polymorphism by RFLP (18):

Genotyping of *MATN3* rs8176070 (SNP6) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

### DNA extraction

DNA was extracted using the Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific Inc; Catalog number: #K0781, Lot: 00242578, California, USA)

### *MATN3* genotyping

PCR was performed in a reaction volume of 50  $\mu$ L using Dream Taq Green PCR Master Mix (Thermo Fisher Scientific Inc; Catalog number: #K1080 California, USA) (#K1080) with 25  $\mu$ L dream taq green PCR master mix, 3  $\mu$ L (5 pmol) forward primer, 3  $\mu$ L (5 pmol) reverse primer, 5  $\mu$ L (10 ng) template DNA, 14  $\mu$ L nuclease-free water.

The PCR primers used were as follows: 5'-d GGACAGGATCCACAAAAAG 3' as a forward primer and 5'-d GAAAGAGGGGCTACAACAGG 3' as a reverse primer (Biosearch Technologies, California, USA).

The amplification protocol was as follows: initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, extending at 72°C for 1 min, and final extension at 72°C for 10 min in a thermocycler (Thermo Scientific PikoReal RealTime PCR System; California, USA). The resultant PCR products showed a single fragment at 501 bp by gel electrophoresis. In total, 10  $\mu$ L of 501-bp product were digested with 10 units of BSEYI restriction enzyme (NEB-R0635S, Lot:0181412 USA) at 37°C for 10 h. Digestion products were visualized on a 2.5% agarose gel containing 2% ethidium bromide. RFLPs were coded as Bb, where

the uppercase letter signifies the absence of the restriction site and the lowercase letter signifies the presence of the site. The wild-type genotype (CC), coded as bb, produced a double band at 149 and 352 bp; heterozygotes (CN), coded as Bb, produced three bands at 501, 149, and 352 bp; and homozygote polymorphic genotype (NN), coded as BB, produced only one band at 501 bp.

### Statistical analysis

The Statistical Package of Social Sciences v. 16.0 (SPSS Inc.; Chicago, IL, USA) computer program was used.

Descriptive statistics were calculated for the data in the form of mean and standard deviation (Mean $\pm$ SD) for quantitative data. Qualitative data were expressed as number and percentage.

### Analytical statistics

In statistical comparison between different groups, the statistical significance was defined as  $p \leq 0.05$  (\*), while a p-value of  $>0.05$  was insignificant. A p-value of  $<0.001$  was considered highly significant (\*\*).

In the studied groups, the representativeness of alleles and genotypes was estimated by the Hardy-Weinberg equilibrium (HWE) by comparing the observed and expected frequencies of genetic variants. Logistic regression analysis was applied to examine association between SNP variants and the risk of OA. The differences in genotype and allele distributions were analyzed by the chi-square ( $\chi^2$ ) test and represented by odds ratios (ORs) and 95% confidence intervals (CIs). A p-value of  $<0.05$  was considered statistically significant at a confidence interval (CI) of 95%.

## Results

The present study included 50 Egyptian patients with primary knee OA (Group I) and 50 apparently healthy volunteers (Group II).

Application of HWE revealed that genotypes of the *MATN3* gene SNP6 (rs8176070) in both cases and control subjects were independent (i.e., they were in HWE). There was no evidence to reject the assumption of HWE in the sample ( $p > 0.001$  for each).

Table 1 presents the characteristics of the studied groups

No significant differences between patients and controls were found with regard to age, sex distribution, and BMI ( $p = 0.28, 0.14$  and  $0.9$  respectively).

**Table 1.** Characteristics of the studied groups

Parameter	Cases (n=50) (Group I)	Control (n=50) (Group II)	p
Sex			
Males	18 (36)	13 (26)	0.28
Females	32 (64)	37 (74)	
Age/years (X±SD)	60.36±6.38	58.48±6.17	0.14
Age groups			0.644
≤65	37 (74)	39 (78)	
>65	13 (26)	11 (22)	
BMI in kg/m <sup>2</sup> (X±SD)	22±2.16	21.98 ±2.77	0.9

BMI: Body Mass Index; p>0.05 was statistically insignificant

**Table 2.** Distribution of *MATN-3* gene SNP6 (rs8176070) genotypes and alleles between case and control groups

	Cases (n=50) (Group I)	Control (n=50) (Group II)	p	OR (95% CI)
<b>Genotype (No. &amp; %)</b>				
• bb	12 (24)	6 (12)	0.564	1.397 (0.447-4.367)
• Bb	30 (60)	20 (40)	0.046*	2.250 (1.011-5.008)
• BB	8 (16)	24 (48)	0.012*	0.342 (0.146-0.803)
<b>Alleles (No. &amp; %)</b>				
• b	54 (54)	32 (32)	0.042	0.552 (0.311-0.982)
• B	(46)46	68 (68)		

\*significant

**Table 3.** Relation between L-index and genotypes in primary knee OA patients

Genotypes	BB (N=8)	Bb (N=30)	bb (N=12)	p
Mean ±SD	3.2±2.25	11.41±2.91	6.67±2.54	0.001**
Good (<10)	8 (100%)	7 (23.3%)	8 (66.7%)	0.044**
Poor (≥10)	0 (0)	23 (76.7%)	4 (33.3%)	

N: number; L-index: Lequesne Algofunctional Index Score; p<0.001 was considered highly significant\*\*

**Table 4.** Relation between KL grade and genotypes in patients with knee OA

Genotypes	BB (N=8)	Bb (N=30)	bb (N=12)	p
KL grade				
- Mild (1-2)	8 (100%)	5 (16.7%)	7 (58.3%)	0.001**
- Severe (3-4)	0 (0.0)	25 (83.3%)	5 (41.7%)	
*1	5 (62.5%)	0 (0.0%)	2 (16.7%)	0.049*
*2	3 (37.5%)	5 (16.7%)	5 (41.7%)	
*3	0 (0.0)	14 (46.7%)	3 (25%)	
*4	0 (0.0%)	11 (36.7%)	2 (16.6%)	

N: number; Kellgren-Lawrence grade, p<0.05 that was considered statistically significant (\*), while p<0.001 was considered highly significant (\*\*)

Table 2 lists the genotyped and allele distributions of the *MATN-3* gene (SNP6rs8176070) for the cases and controls.

A significant difference was found between OA patients and controls with regard to genotypes B**b** and b allele (p=0.046 and 0.042 respective-

ly), with a high risk to develop primary knee OA (OR=2.250; 95% CI=1.011-5.008). On the other hand, the B**B** genotype and B allele were significantly associated with a protective effect against the development of OA (OR=0.342; 95% CI=0.146-0.803). However, the b**b** genotype was associated with a lower risk to develop knee OA (OR=1.397).

As shown in Table 3, with regard to the relation between the L-index score of severity and genotypes in patients with primary knee OA, there was a high statistically significant difference (p<0.001\*\*) in the mean L-index score, with the score being higher in the B**b** genotype with a mean±SD of 11.41±2.91. Patients with b**b** and B**B** genotypes had a mild grade according to the Lequesne score (Mean±SD 6.67±2.54 and 3.2±2.25, respectively).

The relation between KL grade and genotypes in primary knee OA patients is represented in Table 4.

In this study, 25 patients (83.3%) with the B**b** genotype had severe KL grades, with a highly significant difference compared with those with BB and bb genotypes (p<0.001\*\*). Patients with the B**B** genotype had mild KL (100%)-grading OA, while patients the b**b** genotype had mild and severe KL-grading OA. This means that patients with the B**b** genotype had worse radiological findings than those with B**B** and b**b** genotypes.

## Discussion

Cartilaginous tissues contain a considerable amount of *MATN-3* protein, which plays a vital role in the configuration of the collagen (collagen IX)-dependent network, linking cells together, and the collagen-independent pericellular network(19).

The present case-control study investigated the possible association and clinical significance of *MATN-3* gene rs8176070 (SNP6) in Egyptian patients with primary knee OA.

Our analysis revealed that with regard to the genotype B**b** and b allele of *MATN-3* gene SNP6 (rs8176070), a significant difference was found between OA patients and controls (p=0.046 and 0.042 respectively), with a high risk to develop OA (OR=2.250; 95% CI=1.011-5.008).

Previous studies have investigated the relationship between *MATN-3* polymorphism and hand OA or knee OA (11, 13, 20). Two articles have claimed that the SNP6 variation is associated with hand OA but not knee OA (13, 20),

while another article has claimed that SNP6 mutation is not associated with OA (11).

Gu et al. (10) who studied 732 OA cases from China (age range: 48-89 years) found that the B**V** genotype increased the risk of OA (OR=1.724, 95% CI=1.071-2.770; p=0.025), particularly knee OA. They also found that compared with control subjects, the B allele may have an effect on increased knee OA (OR=3.143, 95% CI=2.283-4.328; p=0.000) (10). This may be explained by ethnic differences related to geographic distribution.

The present study also revealed a higher Lequesne score and KL grading of knee OA patients with the B**b** genotype, while patients with the b**b** genotype showed less severe disability and radiological severity than those with the B**V** genotype. Patients with the B**B** genotype had the lowest Lequesne score for disability, and their radiological findings were the mildest. This means that patients with the B**b** genotype had worse clinical and radiological findings than other those with the B**B** and b**b** genotypes.

In a normal human cartilage, Pullig et al. (11) found low but significant expression of *MATN-3* mRNA, which is extremely upregulated in an osteoarthritic cartilage and correlated with disease severity. They stated that *MATN-3* could be used as an indicator of OA progression (21).

*In vitro* studies of *MATN-3* identified its anti-anabolic and pro-catabolic functions, suggesting that it plays an important role in OA (22, 23). Some researchers have highlighted the role of *MATN3* in cartilage homeostasis; hence, its genetic ablation in mice resulted in the development of OA in adulthood without any observable developmental defect (24). It has been found that enhanced *MATN-3* gene and protein expression correlates with the degree of tissue damage that occurs in OA patients (21).

*Matrilin-3* was previously studied by Belluocchio et al. (22); they found that the matrix *MATN-3* molecule was expressed in every cartilage type. In addition, their experimental cell culture recommended that *MATN-3* may represent a useful indicator of the state of differentiation of cells obtained from the articular cartilage (22).

In contrast, Minafra et al. (23) assessed the potential associations of polymorphisms in the main OA susceptibility genes such as *FRZB rs288326 & rs7775*, *ASPN D14 repeats*, *MATN3 rs77245812*, *DVWA rs11718863*, *GDF5 rs143383*, and *PTHR2 rs76758470* with age, clinical fea-

tures such as the American Knee Society score (AKSS), and KL grading in Sicilian individuals. They reported a statistically significant association only between alterations in *GDF5 rs143383 & DVWA rs11718863* genes and KL grading. The *DVWA rs11718863* gene had a predictive role in OA progression because it was associated with a more severe radiographic grade.

Two studies conducted by Vincourt et al. (25, 26) revealed higher levels of *MATN-3* in both the serum and synovial fluid of OA patients.

*Matrilin-3* plays an important role in promoting ECM anabolism as it is considered as an adaptor protein; its function changes from an anabolic effect with a very low concentration range (100 to 200 ng/mL) to a catabolic effect when its levels change to a supra-physiological range (5 to 50 µg/mL) (25). This may be related to a negative feedback mechanism (21).

Jayasurya et al. (27) noticed that even in the presence of interleukin-1β (IL-1β), a concentration of 100 and 200 ng/mL of the soluble recombinant *MATN-3* in chondrocytes stimulates the induction of an interleukin 1 receptor antagonist (IL-1Ra), limits inflammation, and maintains the tensile and elastic strength of cartilaginous tissues through the enhancement of collagen II and aggrecan. On the other hand, it reduces disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS5) through IL-1Ra. Further, in chondrocytes, *MATN-3* downregulates matrix metalloproteinase-13 (MMP-13); thus, it plays a role in the inhibition of inflammation-induced hypertrophy (27).

In contrast, Klatt et al. (28) reported that enhanced production of MMP1, MMP3, MMP13, IL-1β, IL-6, IL-8, iNOS, and COX-2 in primary human chondrocytes was induced by high concentrations (5 to 50 µg/mL) of *MATN-3* with consequently increased inflammation and catabolism of ECM. In addition, elevated levels of the free form of *MATN-3* resulting from the degradation of ECM were induced by the overexpression of ADAMTS4 and ADAMTS5 (28).

In conclusion, the investigated polymorphism in the *MATN-3* gene may reflect the risk and severity of knee OA in the Egyptian population, particularly with the B**b** genotype.

#### Limitations

Because of the relatively small sample size, these results require to be confirmed by further studies in different populations with a large sample size to highlight the possible relation between the *MATN3* gene and OA.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Benha University School of Medicine, Benha, Egypt.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

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